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Human Papillomavirus-Type Distribution in Women With and Without Cervical Neoplasia in North India

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Abstract

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Our objective was to determine the human papillomavirus (HPV)-type prevalence in cervical samples in women with and without cervical neoplasia in an opportunistic hospital-based cancer-screening program.

A cross-sectional study of 524 women presenting from January 2003 through June 2005 with symptoms of persistent vaginal discharge, intermenstrual bleeding, and postcoital bleeding or detected to have an unhealthy cervix underwent HPV genotyping by consensus polymerase chain reaction and reverse line-blot hybridization assay, conventional Pap smear, and colposcopy, with directed biopsy from all lesions detected.

The prevalence rates of HPV infection among women with normal, low-grade cervical neoplasia (CIN 1) and high-grade CIN (>CIN2) were found to be 7.6%, 42.3%, and 87.5%, respectively. Seventeen high-risk and 6 low-risk HPV types were identified by the reverse line-blot assay. Multiple infections were seen in 20% of women. In normal women, the 6 commonest types were HPV-16, HPV-89, HPV-39, HPV-52, HPV-62, and HPV-18, whereas in high-grade disease, these were all high-risk types HPV-16, HPV-18, HPV-33, HPV-39, HPV-35, and HPV-56. HPV-16 was the commonest type in all groups, seen in 49.4% cases overall and in 74.3% of high-grade squamous intraepithelial lesion. It was followed by HPV-18 (7.4%) and HPV-33 and HPV-39 (4.9% each). HPV-89 was the commonest low-risk type (9.9%). HPV-16/18 were associated with 34.3% of normal, 45.4% of low-grade and 65.7% of high-grade lesions. A wide spectrum of HPV types is seen in north Indian women, with the majority being HPV-16 in all grades of histology. A vaccine against HPV-16 and HPV-18 could prevent two thirds of cases of high-grade cervical neoplasia.

Cancer of the cervix uteri is the second most common cancer among women worldwide and the commonest cancer among women in India (1). The causal role of human papillomavirus (HPV) in all cancers of the uterine cervix has been established (2). It has been difficult to implement effective cytology-based screening programs in resource-poor settings because of cost and infrastructure requirements, false-negative results of Pap tests, and underscreening of populations at risk (3). The recent availability of a prophylactic vaccine has made control of cervical cancer a foreseeable reality (4). However, there are only limited data available from India on HPV-type prevalence tested by standardized protocols designed to determine multiple HPV types and infections in cervical intraepithelial neoplasia (CIN) and invasive cervical cancer (5-8). The present study aims to determine HPV types present in cervical samples from a population of north Indian women who presented at a hospital with gynecological symptoms.

MATERIALS AND METHODS

This cross-sectional study was carried out in the gynecology outpatient department from January 2003 through June 2005. Women presenting with complaints of persistent vaginal discharge (≥ 6 mo), irregular menstrual bleeding, and postcoital bleeding or those found on examination to have an unhealthy cervix were invited to participate in an opportunistic cancer-screening program. Exclusion criteria included the following: younger than 30 years, unmarried, hysterectomized, previous surgical procedures on cervix, gross tumor on the cervix, and pregnancy. Informed written consent was taken from the women. Ethical

clearance was obtained from the Institutional Review Board. A total of 625 women were recruited; 546 (87.4%) eligible women were enrolled and an enrolment questionnaire was completed.

Clinical Examination and Investigation

All patients underwent conventional Pap smear, cervical sampling for HPV DNA testing, colposcopy, and biopsy from all lesions with a Reid score ≥ 0 . Pap smear was taken with an Ayre spatula and endocervical brush; next, a cervical brush sampler (Digene, Gaithersburg, MD) was introduced inside the endocervix, with the lowermost bristles touching the ectocervix. The brush was rotated 3 to 5 times in the counterclockwise direction and placed in the Digene specimen collection tube. All women underwent colposcopic examination by an experienced gynecologist.

Sample Storage, Processing, and HPV Testing

The samples collected in Digene Specimen Transport Medium were stored at -70°C until further processing. The sample was processed as previously described (9,10). In brief, 150 μL of the sample was digested with 15 μL of $10\times$ digestion buffer (containing 700 μL of 20 mM Tris-HCl—1 mM ethylene diaminetetraacetic acid (TE) buffer, 100 μL 10% Tween-20, and 200 μL of 20 mg/mL proteinase K) at 65°C for 1 hour, followed by heat inactivation at 95°C for 10 minutes. The DNA was precipitated with ethanol and ammonium acetate at -20°C overnight. After centrifugation at $21,000\times g$ for 30 minutes at 4°C for pelleting the DNA, the pellet was dried, resuspended in 75 μL of TE, and stored at -20°C until amplification for HPV testing. The specimen DNA was amplified using PGMY 09/11 HPV-specific primers that amplify the 450-bp fragment of L1 open reading frame of genital HPV. Human [beta]-globin target was coamplified with HPV consensus primers to determine adequacy of the specimen. The polymerase chain reaction (PCR) products were denatured and hybridized to an immobilized HPV probe array on strips (kind gift of Roche Molecular Systems, Alameda, CA). Positive hybridization was detected by color precipitation at the probe site and the type determined by reading from a reference overlay. Each amplification run included HPV DNA-positive controls (SiHa cell line/HeLa cell line) as well as no HPV DNA-negative controls.

For analysis purposes, samples were considered sufficient for HPV determination if the [beta]-globin probe was detected. All [beta]-globin-negative samples were excluded from further analysis. HPV types were grouped into high-risk and low-risk types as follows: high-risk (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 69, 70, 73, 82, and its subtype IS039) and low-risk (6, 11, 40, 42, 54, 55, 57, 61, 62, 64, 71, 72, 83, 84, and 89) (11). Women were classified as normal, low-grade disease, and high-grade disease based on histological diagnosis. In cases with discordance between cytology and histology, loop electrosurgical excision procedure (LEEP)/conization was done and the worse grade of histology was taken into consideration.

RESULTS

Of the 546 women enrolled, 10 women did not complete the study protocol and PCR was missing in 9 cases. In 3 samples, [beta]-globin could not be amplified, so it was not possible to comment on the presence and type of HPV. Data were thus evaluated for 524 cases. The demographic characteristics were as follows: age range was from 30 to 74 years (median 36 years), most (72.6%) were in the age group of 30 to 40 years; 39.2% of the women had no formal education; 26.0% had received some primary education; and 23.7% of women had received high school or higher education. Most women came from Delhi and other states of north India and belonged to lower (47.2%) and middle (49.1%) socioeconomic class; 35.1% reported 4 or more births. The mean age at first coitus was 19 ± 3.3 years.

Prevalence of HPV Infection

Polymerase chain reaction detected HPV infection in 81 women (15.5%), of whom 69 (13.2%) had high-risk HPV (hrHPV) infection. A total of 101 infections were seen in 81 women. The reverse line-blot assay identified (17) high-risk HPV types; in order of frequency, these were HPV-16, HPV-18, HPV-33, HPV-39, HPV-52, HPV-58, HPV-53, HPV-51, HPV-35, HPV-73, HPV-31, HPV-56, HPV-70, HPV-66, HPV-82, HPV-68, and HPV-59. Six low-risk types were identified, that is, HPV-89, HPV-62, HPV-84, HPV-55, HPV-42, and HPV-72. HPV-16 was the most prevalent, seen in 49.4% of all HPV-positive cervical samples and in 74.3% of high-grade squamous intraepithelial lesion (SIL). HPV-18 was seen in 6 cases and HPV-33 and HPV-39 in 4 cases each. HPV-89 was the commonest low-risk type, seen in 8 cases. Multiple infections with high-risk types were seen in 16 cases, 5 of these were a single high-risk type in association with a low-risk type, whereas 2 had >1 hrHPV in association with low-risk infection. There were 12 cases of infection with only low-risk types, 1 of them multiple. Two women had coinfection with 3 high-risk types each.

Pap smear showed atypical squamous cells of undetermined significance in 23 cases, atypical squamous cells—cannot exclude HSIL in 8, and atypical glandular cells of undetermined significance in 4 cases. LEEP was done in 21 and conization in 9 cases of discordance between cytology, colposcopy, and biopsy. Histologically proven CIN or invasive cancer was present in 66 women (CIN1, 26; CIN2, 13; CIN3, 19; invasive cancer, (8). Table 1 shows the HPV-type distribution with respect to histopathology. Among normal women, HPV DNA was detected in 7.6% of histologically normal women and infection with high-risk types accounted for 70.6% of them. Among women with CIN 1, HPV DNA was detected in 42.3% and infection with high-risk types accounted for 81.8%. Among women with high-grade disease (CIN2, CIN3, or invasive cancer), HPV DNA was detected in 87.5% and infection with high-risk types accounted for 100%. This group included 8 cases of invasive cancer that did not have any obvious tumor on naked-eye examination. The HPV types in these 8 cases were HPV-16 (5), HPV-18 (1), HPV-33 (1), and HPV negative (1).

HPV-16 was the commonest type in each group, although its proportion increased with increasing grade of disease (fig. 1). There was no correlation of age with the prevalence of HPV-16 or HPV-89 (the commonest high- and low-risk types, respectively).

DISCUSSION

This study was conducted in an opportunistic setting in a tertiary hospital that receives a mixed population of women, some of whom are self-referred, whereas others are referred by their health-care providers for specialist care. Most women enrolled in this study were from Delhi and neighboring states of north India. Because these women were symptomatic, a higher prevalence of HPV DNA positivity as well as of CIN was expected. In keeping with this hypothesis, the overall prevalence of HPV DNA was found to be 15.5%, and high-grade disease was identified in 7.6% cases.

All participants in the present study underwent conventional cytology, colposcopy, and biopsy. Cases with discordance between any tests underwent LEEP or conization. The worst histology grade was taken into consideration. HPV positivity among women with no cervical disease was 7.6%. This is in keeping with reported literature. The pooled meta-analysis from the International Agency for Research on Cancer reported an overall HPV prevalence of 9.6% among Asian women with no cervical disease (12). It is also comparable with the HPV prevalence we have seen in a study carried out in a rural community in north India (13).

In low-grade disease, HPV positivity was found to be 42.3%, with 18.2% associated with low-risk types only. Very variable prevalence of HPV-positive cases has been reported in the category of low-grade SIL ranging from 29% to 100% (14). In high-grade CIN and invasive cancer, we found 87.5% cases to be HPV positive, and they were all associated with high-risk HPV.

The 6 commonest HPV types seen in each histological group were as follows: normal—HPV-16, HPV-89, HPV-39, HPV-52, HPV-62, and HPV-18; low-grade disease—HPV-16, HPV-89, HPV-33, HPV-35, HPV-52, and HPV-18; and high-grade disease—HPV-16, HPV-18, HPV-33, HPV-39, HPV-35, and HPV-55. A comprehensive meta-analysis of more than 7000 cases of high-grade SIL from studies published up to January 2006 found the commonest HPV types; in decreasing order of prevalence were HPV-16, HPV-31, HPV-58, HPV-18, HPV-33, HPV-52, HPV-35, HPV-51, HPV-56, HPV-45, HPV-39, HPV-66, and HPV-6 (15,16)

HPV-16 was by far the commonest single type in all histological categories. Similar results have been reported in other studies from south India also (7,17), although 1 study from a rural community in south India found HPV-52 to be the commonest of all infections (29.4%) followed by HPV-16 in 17.6% (5). We did not see any case of HPV-45 in this study, but among cases with invasive cancer, we have previously found it to be the third commonest after HPV-16 and HPV-18 (8). Interestingly, although HPV-6 is commonly reported in literature, no case of low-risk HPV-6 or HPV-11 was found in the present study. In our previous report on invasive cancers, we had seen only 1 case each of these types, in association with hrHPV (8). The comparatively low prevalence of HPV-6 and HPV-11 has been reported in other studies also (5,7). It is also consistent with the clinical observation that we see benign warts in very few patients. The commonest low-risk type seen in our

study was HPV-89. Low-risk types were seen mainly in normal women and those with low-grade disease.

In our previous study on invasive cancers, we had reported that a vaccine against HPV-16 and HPV-18 would be able to protect against 76.4% of cancers (8). In the present study, we find that such a vaccine would protect against 65.7% of cases of high-grade cervical neoplasia that were associated only with HPV-16 and HPV-18 and not associated with other coinfections. However, it is possible that cross-protection may increase the proportion of women who could benefit from this protection.

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FIG. 1.

Human papilloma virus (HPV) prevalence among women with no, low-grade, and high-grade cervical neoplasia on histology. LSIL indicates low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

HPV-type distribution in relation to histological grade *No cervical abnormality on colposcopy/histology.+Low-risktype.CIN indicates cervical neoplasia; HPV, human papillomavirus; N, total number of cases per group; n, number of positive for HPV DNA.

TABLE 1

HPV type	Normal* (N = 458, n = 35, 7.6%)		Low-Grade Disease (CIN1) (N = 26, n = 11, 42.3%)		High-Grade Disease (CIN2) (N = 40, n = 35, 87.5%)	
	No. (%)	HPV Type	No. (%)	HPV Type	No. (%)	HPV Type
Single infection						
16	9 (25.7%)	16	4 (36.4%)	16	20 (57.1%)	
39	2 (5.7%)	33	1 (9.1%)	18	3 (16.3%)	
18	2 (5.7%)	35	1 (9.1%)	33	3 (16.3%)	
58	2 (5.7%)	52	1 (9.1%)	39	1 (2.9%)	
31	1 (2.9%)	89†	2 (18.2%)			
51	1 (2.9%)					
53	1 (2.9%)					
70	1 (2.9%)					
73	1 (2.9%)					
89†	5 (14.3%)					
84†	2 (5.7%)					
42†	1 (2.9%)					
62†	1 (2.9%)					
Multiple infections						
39,62†	1 (2.9%)	18,66,62†	1 (9.1%)	16,62†	1 (2.9%)	
52,55†	1 (2.9%)	51,52,82	1 (9.1%)	16,35	1 (2.9%)	
52,89†	1 (2.9%)			16,55†	1 (2.9%)	
16,66	1 (2.9%)			16,56	1 (2.9%)	
53,56	1 (2.9%)			16,68	1 (2.9%)	
62†,72†	1 (2.9%)			16,73	1 (2.9%)	

Normal* (N = 458, n = 35, 7.6%)		Low-Grade Disease (CIN1) (N = 26, n = 11, 42.3%)		High-Grade Disease (CIN2) (N = 40, n = 35, 87.5%)	
HPV type	No. (%)	HPV Type	No. (%)	HPV Type	No. (%)
Single infection					
				51,82,84 [†]	1 (2.9%)
				31,53,59	1 (2.9%)

CIN indicates cervical neoplasia; HPV, human papillomavirus; N, total number of cases per group; n, number of positive for HPV DNA.

* No cervical abnormality on colposcopy/histology.

[†] Low-risk type.